

Effects of Bacillus Serine Proteases on the Bacterial Biofilms

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Abstract

© 2017 Olga Mitrofanova et al. *Serratia marcescens* is an emerging opportunistic pathogen responsible for many hospital-acquired infections including catheter-associated bacteremia and urinary tract and respiratory tract infections. Biofilm formation is one of the mechanisms employed by *S. marcescens* to increase its virulence and pathogenicity. Here, we have investigated the main steps of the biofilm formation by *S. marcescens* SR 41-8000. It was found that the biofilm growth is stimulated by the nutrient-rich environment. The time-course experiments showed that *S. marcescens* cells adhere to the surface of the catheter and start to produce extracellular polymeric substances (EPS) within the first 2 days of growth. After 7 days, *S. marcescens* biofilms mature and consist of bacterial cells embedded in a self-produced matrix of hydrated EPS. In this study, the effect of *Bacillus pumilus* 3-19 proteolytic enzymes on the structure of 7-day-old *S. marcescens* biofilms was examined. Using quantitative methods and scanning electron microscopy for the detection of biofilm, we demonstrated a high efficacy of subtilisin-like protease and glutamyl endopeptidase in biofilm removal. Enzymatic treatment resulted in the degradation of the EPS components and significant eradication of the biofilms.

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References

- [1] J. Wingender, T. R. Neu, and H. Flemming, "What are Bacterial Extracellular Polymeric Substances?" *Microbial Extracellular Polymeric Substances*, pp. 1-19, 1999.
- [2] H.-C. Flemming, T. R. Neu, and D. J. Wozniak, "The EPS matrix: the 'House of Biofilm Cells'," *Journal of Bacteriology*, vol. 189, no. 22, pp. 7945-7947, 2007.
- [3] H. Flemming, "EPS - Then and Now," *Microorganisms*, vol. 4, no. 4, p. 41, 2016.
- [4] S. L. Chua, J. K. H. Yam, P. Hao et al., "Selective labelling and eradication of antibiotic-Tolerant bacterial populations in *Pseudomonas aeruginosa* biofilms," *Nature Communications*, vol. 7, Article ID 10750, 2016.
- [5] T. Song, M. Duperthuy, and S. Wai, "Sub-Optimal Treatment of Bacterial Biofilms," *Antibiotics*, vol. 5, no. 2, 23 pages, 2016.
- [6] H. Flemming and J. Wingender, "The biofilm matrix," *Nature Reviews Microbiology*, vol. 8, no. 9, pp. 623-633, 2010.
- [7] Y. Ding, Y. Zhou, J. Yao et al., "In Situ Molecular Imaging of the Biofilm and Its Matrix," *Analytical Chemistry*, vol. 88, no. 22, pp. 11244-11252, 2016.
- [8] R. M. Donlan, "Biofilms: microbial life on surfaces," *Emerging Infectious Diseases*, vol. 8, no. 9, pp. 881-890, 2002.
- [9] H. Pillich, M. Puri, and T. Chakraborty, "ActA of *Listeria monocytogenes* and its manifold activities as an important listerial virulence factor," *Current Topics in Microbiology and Immunology: The Actin Cytoskeleton and Bacterial Infection*, pp. 113-132, 2016.

- [10] I. Randrianjatovo-Gbalou, P. Rouquette, D. Lefebvre, E. Girbal-Neuhausser, and C. Marcato-Romain, "In situ analysis of *Bacillus licheniformis* biofilms: amyloid-like polymers and eDNA are involved in the adherence and aggregation of the extracellular matrix," *Journal of Applied Microbiology*, vol. 122, no. 5, pp. 1262-1274, 2017.
- [11] P. Baker, P. J. Hill, B. D. Snarr et al., "Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent *Pseudomonas aeruginosa* biofilms," *Science Advances*, vol. 2, no. 5, pp. e1501632-e1501632, 2016.
- [12] M. S. Blackledge, R. J. Worthington, and C. Melander, "Biologically inspired strategies for combating bacterial biofilms," *Current Opinion in Pharmacology*, vol. 13, no. 5, pp. 699-706, 2013.
- [13] J. B. Kaplan, C. Ragunath, N. Ramasubbu, and D. H. Fine, "Detachment of *Actinobacillus actinomycetemcomitans* biofilm cells by an endogenous β -hexosaminidase activity," *Journal of Bacteriology*, vol. 185, no. 16, pp. 4693-4698, 2003.
- [14] T. Iwase, Y. Uehara, H. Shinji et al., "Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization," *Nature*, vol. 465, no. 7296, pp. 346-349, 2010.
- [15] J.-H. Park, J.-H. Lee, M. H. Cho, M. Herzberg, and J. Lee, "Acceleration of protease effect on Staphylococcus aureus biofilm dispersal," *FEMS Microbiology Letters*, vol. 335, no. 1, pp. 31-38, 2012.
- [16] C. Longhi, G. L. Scoarughi, F. Poggiali et al., "Protease treatment affects both invasion ability and biofilm formation in *Listeria monocytogenes*," *Microbial Pathogenesis*, vol. 45, no. 1, pp. 45-52, 2008.
- [17] A. Khanna, M. Khanna, and A. Aggarwal, "Serratia marcescens rare opportunistic nosocomial pathogen and measures to limit its spread in hospitalized patients," *Journal of Clinical and Diagnostic Research*, vol. 7, no. 2, pp. 243-246, 2013.
- [18] H. J. Yoon, J. Y. Choi, Y. S. Park et al., "Outbreaks of Serratia marcescens bacteriuria in a neurosurgical intensive care unit of a tertiary care teaching hospital: a clinical, epidemiologic, and laboratory perspective," *American Journal of Infection Control*, vol. 33, no. 10, pp. 595-601, 2005.
- [19] W. A. Rutala, V. A. Kennedy, H. B. Loflin, and F. A. Sarubbi Jr., "Serratia marcescens nosocomial infections of the urinary tract associated with urine measuring containers and urinometers," *The American Journal of Medicine*, vol. 70, no. 3, pp. 659-663, 1981.
- [20] T. Yamamoto, A. Ariyoshi, and K. Amako, "Fimbria-Mediated Adherence of Serratia marcescens Strain US5 to Human Urinary Bladder Surface," *Microbiology and Immunology*, vol. 29, no. 7, pp. 677-681, 1985.
- [21] N. Takata, H. Suganaka, S. Kotani, M. Ogawa, and G. Kosaki, " β -Lactam resistance in Serratia marcescens: Comparison of action of benzylpenicillin, apalcillin, cefazolin, and ceftizoxime," *Antimicrobial Agents and Chemotherapy*, vol. 19, no. 3, pp. 397-401, 1981.
- [22] R. Coria-Jiménez and C. Ortiz-Torres, "Aminoglycoside resistance patterns of Serratia marcescens strains of clinical origin," *Epidemiology and Infection*, vol. 112, no. 1, pp. 125-131, 1994.
- [23] L. Alberti and R. M. Harshey, "Differentiation of Serratia marcescens 274 into swimmer and swarmer cells," *Journal of Bacteriology*, vol. 172, no. 8, pp. 4322-4328, 1990.
- [24] H. Matsumoto, T. Tazaki, and S. Hosogaya, "A Generalized Transducing Phage of Serratia marcescens," *Japanese Journal of Microbiology*, vol. 17, no. 6, pp. 473-479, 1973.
- [25] G. D. Christensen, W. A. Simpson, J. J. Younger et al., "Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices," *Journal of Clinical Microbiology*, vol. 22, no. 6, pp. 996-1006, 1985.
- [26] S. Stepanović, D. Vuković, V. Hola et al., "Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci," *APMIS*, vol. 115, no. 8, pp. 891-899, 2007.
- [27] C. Reichhardt, A. N. Jacobson, M. C. Maher et al., "Congo red interactions with curli-producing E. coli and native curli amyloid fibers," *PLoS ONE*, vol. 10, no. 10, Article ID e0140388, 2015.
- [28] U. Gophna, M. Barlev, R. Seijffers, T. A. Oelschlager, J. Hacker, and E. Z. Ron, "Curli fibers mediate internalization of Escherichia coli by eukaryotic cells," *Infection and Immunity*, vol. 69, no. 4, pp. 2659-2665, 2001.
- [29] L. A. Lyublinskaya, T. L. Voyushina, and V. M. Stepanov, "Enzymatic synthesis of serine proteases peptide substrates," *Russian Journal of Bioorganic Chemistry*, vol. 8, no. 12, pp. 1620-1624, 1982.
- [30] I. B. Leshchinskaya, E. V. Shakirov, E. L. Itskovitch et al., "Glutamyl endopeptidase of Bacillus intermedius, strain 3-19," *FEBS Letters*, vol. 404, no. 2-3, pp. 241-244, 1997.
- [31] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding," *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248-254, 1976.
- [32] G. Di Bonaventura, S. Stepanović, C. Picciani, A. Pompilio, and R. Piccolomini, "Effect of environmental factors on biofilm formation by clinical Stenotrophomonas maltophilia isolates," *Folia Microbiologica*, vol. 52, no. 1, pp. 86-90, 2007.

- [33] A. Rochex and J.-M. Lebeault, "Effects of nutrients on biofilm formation and detachment of a *Pseudomonas putida* strain isolated from a paper machine," *Water Research*, vol. 41, no. 13, pp. 2885-2892, 2007.
- [34] S. A. Rice, K. S. Koh, S. Y. Queck, M. Labbate, K. W. Lam, and S. Kjelleberg, "Biofilm formation and sloughing in *Serratia marcescens* are controlled by quorum sensing and nutrient cues," *Journal of Bacteriology*, vol. 187, no. 10, pp. 3477-3485, 2005.
- [35] J. Van Gestel, H. Vlamakis, and R. Kolter, "Division of labor in biofilms: The ecology of cell differentiation," *Microbiology Spectrum*, vol. 3, no. 2, Article IDMB-0002-2014, 2015.
- [36] R. Daniels, J. Vanderleyden, and J. Michiels, "Quorum sensing and swarming migration in bacteria," *FEMS Microbiology Reviews*, vol. 28, no. 3, pp. 261-289, 2004.
- [37] J. S. Webb, L. S. Thompson, S. James et al., "Cell death in *Pseudomonas aeruginosa* biofilm development," *Journal of Bacteriology*, vol. 185, no. 15, pp. 4585-4592, 2003.
- [38] A. Taglialegna, I. Lasa, and J. Valle, "Amyloid structures as biofilm matrix scaffolds," *Journal of Bacteriology*, vol. 198, no. 19, pp. 2579-2588, 2016.
- [39] Y. M. Kirillova, E. O. Mikhailova, N. P. Balaban et al., "Biosynthesis of the *Bacillus intermedius* subtilisin-like serine proteinase by the recombinant *Bacillus subtilis* strain," *Microbiology*, vol. 75, no. 2, pp. 142-147, 2006.
- [40] E.O.Mikhailova, A.M.Mardanov, N. P. Balaban, G.N. Rudenskaya, and M. R. Sharipova, "Isolation and characterization of a subtilisin-like proteinase of *Bacillus intermedius* secreted by the *Bacillus subtilis* recombinant strain AJ73 at different growth stages," *Biochemistry (Moscow)*, vol. 72, no. 2, pp. 192-198, 2007.
- [41] T. R. Shamsutdinov, N. P. Balaban, A. M. Mardanov, Y. V. Danilova, G. N. Rudenskaya, and M. R. Sharipova, "Isolation and characteristics of *Bacillus intermedius* glutamyl endopeptidase secreted by a recombinant strain of *Bacillus subtilis* at various growth phases," *Russian Journal of Bioorganic Chemistry*, vol. 34, no. 3, pp. 290-293, 2008.
- [42] Y. V. Danilova, E. I. Shagimardanov, A. B. Margulis et al., "Bacterial enzymes effectively digest Alzheimer's β -amyloid peptide," *Brain Research Bulletin*, vol. 108, pp. 113-117, 2014.
- [43] Y. Lequette, G. Boels, M. Clarisse, and C. Faille, "Using enzymes to remove biofilms of bacterial isolates sampled in the food industry," *Biofouling*, vol. 26, no. 4, pp. 421-431, 2010.
- [44] J. A. Jurcisek and L. O. Bakaletz, "Biofilms formed by nontypeable *Haemophilus influenzae* in vivo contain both doublestranded DNA and type IV pilin protein," *Journal of Bacteriology*, vol. 189, no. 10, pp. 3868-3875, 2007.
- [45] P. M. Gallo, G. J. Rapsinski, R. P. Wilson et al., "Amyloid-DNA Composites of Bacterial Biofilms Stimulate Autoimmunity," *Immunity*, vol. 42, no. 6, pp. 1171-1184, 2015.
- [46] L. Selan, F. Berlutti, C. Passariello, M. R. Comodi-Ballanti, and M. C. Thaller, "Proteolytic enzymes: a new treatment strategy for prosthetic infections?" *Antimicrobial Agents and Chemotherapy*, vol. 37, no. 12, pp. 2618-2621, 1993.